REMARKS

Status of the claims

Claims 1-21 are pending in the application. Claims 1, 13 and 21 have been amended herein. No new matter has been added by way of these amendments. As such, entry thereof is respectfully requested.

Objections to the Abstract

The Abstract of the Disclosure has been objected to for failing to appear on a separate sheet. The Abstract has been amended herein to correct this informality.

Objections to the specification

The specification has been objected to for recitation of the trademark Aviclel™. The specification has been amended to capitalize the indicated trademark. Generic terminology was previously recited in the specification for Avicel. Withdrawal of the objection is therefore respectfully requested.

Objections to the claims

Claims 1 and 2 have been objected to for recitation of the abbreviation CBM. Claim 13 has been further objected to for recitation of the trademark Aviclel™. The claims have been amended to address these objections, withdrawal of the objections is therefore respectfully requested.

Double patenting rejection

Claims 1-21 have been rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-22 of USP 7,462,701. Applicants note that the Examiner subsequently references USP 7,396,670, which Applicants believe to be in error. Applicants further traverse this rejection and withdrawal thereof is respectfully requested.

The invention of claims 1-22 of USP '701 essentially correspond to steps (1)-(7) of Figure 1 of the instant specification. The instant invention, on the other hand, essentially pertains to steps (10)-(17) of Figure 1. While a "preferred embodiment" of the instant invention also includes steps (1)-(7), claims 1-22 of USP '701, when considered in view of the USP '701 specifiction, fail to suggest the present invention. There are several notable differences between the instant invention and that of claims 1-22 of USP '701. Briefly,

(i) the fusion protein in USP '701 is solely purified from transgenic plants. However, in the present invention the fusion protein is not limited to such fusion proteins. This is evidenced by the fact that the instant claims do not recite step (a) of the USP '701, i.e. "disrupting the transgenic plant material";

(ii) in addition, the final product of USP '701 claims is the isolated fusion protein (still containing the CBM module). The present claims, on the other hand, specifically deal with the removal of the CBM module from the heterologous fusion protein having a cleavage site.

As such, the invention of instant claims 1-21 and that of claims 1-22 of USP '701 are patentably distinct and withdrawal of the rejection is respectfully requested.

Rejections under 35 U.S.C.§112, 2nd paragraph

Claims 1-21 have been rejected under 35 U.S.C.§112, 2nd paragraph as being indefinite. More specifically claim 1, part (g) has been rejected for lacking antecedent basis for the phrase "substantially to insoluble cell-wall plant material." Claim 1 has been amended to address this issue.

Claim 21 has been rejected for allegedly being unclear in the recitation of "substantial sequence identity". Claim 21 has been amended by replacing the term "substantial sequence identity" with a specific percentage sequence identity. Basis for this amendment is found at least on page 13, line 32. As such, no new matter has been added and withdrawal of the rejection is respectfully requested.

Rejections 35 U.S.C.§112, 1st paragraph

Claims 1-4 and 6-21 have been rejected under 35 U.S.C.§112, 1st paragraph for lacking enablement and sufficient written description. More specifically, the Examiner asserts that the specification is only enabled for a process for the purification of a heterologous protein, which comprises providing a fusion protein of the heterologous protein fused to a CBM of SEQ ID NO:1, a xylanase 10A gene from *Thermotoga maritima* intercepted by a proteolytic cleavage site; and contacting the fusion protein with a functional protease fused to a CBM, wherein the functional protease is a bovine enterokinase, so as to cleave the CBM from the heterologous protein of interest.

The Examiner asserts that the specification is not enabled for nor sufficiently describes a fusion protein comprising any CBM from any source, including a sequence having undefined identity with SEQ ID NO:1, or from any region of the xylanase 10A gene from *Thermotoga maritime* or for contacting the fusion protein with any functional protease fused to a CBM.

Applicants traverse these rejections and withdrawal thereof is respectfully requested. Both the rejection for lack of enablement and the rejection for lack of written description are based on the assertion by the Examiner that the instant claims are directed to the use of **any** CBM.

The present invention is related to a method for the purification of recombinant fusion proteins comprising CBM, wherein the CBM binds reversibly to a polysaccharide matrix. Contrary to the assertion of the Examiner, the instant claims do not encompass "any" CBM, but rather those CBMs which are defined as being "capable of binding reversibly to a polysaccharide matrix and being released from such matrix by nondenaturing elution conditions." This is the final recited feature of claim 1. Claim 1 has been amended herein to precede this feature by a comma and set it apart from step (g) to make it clear that this feature is not "optional", as is step (g).

The specification provides a detailed disclosure of an embodiment of the invention using a CBM from *Thermotoga maritima,* as Examiner rightly notes. Applicants submit that it is well within the realm of the skilled person to test, on the basis of the detailed examples of the specification, whether a CBM, other than that specifically exemplified, will bind reversibly to a polysaccharide matrix or not. One of ordinary skill in the art would know that this could be done by performing a straight-forward binding experiment with a polysaccharide matrix column to test whether or not the CBM is eluted off the column with, e.g., a high concentration of saccharide solution or high ionic strength elution buffer, as described on p. 18, paragraph (7).

Regarding the enablement rejection, Applicants again stress that the claims do not encompass *any* random CBM protein, but rather a CBM from a certain subset; and whether or not a CBM protein is member of the subset of proteins encompassed by the claims can be readily tested as explained above. Further, the Examiner contends that the invention is not enabled for nor sufficiently describes a process involving a CBM in a fusion protein intercepted with a cleavage site, and contacting the fusion protein with *any* protease, to cleave the CBM from the heterologous protein of interest.

Applicants respectfully note that incorporating a suitable cleavage site is simply a straight-forward matter of choice that is readily apparent to one of ordinary skill practicing the invention. In the exemplified embodiment, "enterokinase" is used, which cleaves after lysine if the Lys is preceded by four Asp resides and is not followed by a Pro. The characteristics of the cleavage site for enterokinase are well known to those skilled in the art. Likewise for other commercially useful and readily available proteases, the skilled person would readily know how to design, synthesize and incorporate a particular cleavage site sequence intercepting the parts of the fusion protein. As such, the instant invention as currently claims is fully enabled and described.

Further Applicants are not claiming broadly defined polypeptides, but rather a novel and inventive *process* defined by no less than *seven* clear and concise method steps, making use of certain functionally well-defined biochemical entities. It is unreasonable that Applicant should point out all possible variants of these entities and be limited to those particular entities used to demonstrate the usefulness and practicability of the *process.* It will clear to the skilled person

that, e.g., a multitude of well known proteases can be used in the invention, the choice of which is preferred in a certain application may depend on e.g. the particular heterologous protein being purified and the suitable conditions for that protein, the scale and structure of the biding system (e.g. expanded bed column type or other type).

In this regard, the Examiner is directed to the holding of by the Court of Appeals for the Federal Circuit in *Capon v. Eshhar*, 76 USPQ2d 1078 (Fed. Cir. 2005). In *Capon*, the claims at issue are exemplified in the following claim 1 from "Eshar's '994 application",

> 1. A chimeric gene comprising
>
> a first gene segment encoding a single-chain Fv domain (scFv) of a specific antibody and
>
> a second gene segment encoding partially or entirely the transmembrane and cytoplasmic, and optionally the extracellular, domains of an endogenous protein
>
> wherein said endogenous protein is expressed on the surface of cells of the immune system and triggers activation and/or proliferation of said cells, which chimeric gene, upon transfection to said cells of the immune system, expresses said scFv domain and said domains of said endogenous protein in one single chain on the surface of the transfected cells such that the transfected cells are triggered to activate and/or proliferate and have MHC nonrestricted antibody-type specificity when said expressed scFV domain binds to its antigen.

The claims had been rejected as lacking sufficient written description under 35 U.S.C.§112, 1st paragraph because the claims encompassed "novel genetic material described in terms of the functional characteristics of the protein it encodes." *Capon* at 1082.

The Court of Appeals for the Federal Circuit held that the rejection was in error. In the *Capon* decision, the court noted that the "invention does not concern the discovery of gene function or structure, as in *Lilly*" and that the "chimeric genes here at issue are prepared from known DNA sequences of known function." The court held that it was in error to find that "the specifications do not meet the written description requirement because they do not reiterate the structure or formula or chemical name for the nucleotide sequences of the claimed chimeric genes."

The instant claims are similar to the situation in *Capon* because that present invention is

not directed to new or novel components, but rather an novel and unobvious method using known components.

As such, the instant invention as claimed is both enabled and sufficiently described. Withdrawal of the rejections is therefore respectfully requested.

Rejections under 35 U.S.C.§103

Claims 1-4 and 6-20 have been rejected under 35 U.S.C.§103 as being obvious over Haynes et al. (USP '715) combined with Shani et al. (WO '174). Haynes et al. is asserted to teach an aqueous phase separation or purification system together with methods for their preparation and use. The systems of Hayes et al. are asserted to be based on polymer-ligand conjugates, wherein the polymer is an oligosaccharide and the ligand is an oligosaccharide binding protein, such as CBM. Following binding, the composition is removed from the oligosaccharide using a specific or non-specific protease. The system and methods of Haynes et al. are asserted to differ from the instant invention in failing to disclose the feature that the fusion proteins are expressed in transgenic plants or obtained from transgenic plants.

Shani et al. is asserted to teach a process of expressing a recombinant protein in a plant and a method of isolating the recombinant protein from the plant. The Examiner asserts that it would have been obvious to combine the method of Shani et al. with those of Haynes et al. with the motivation to do so being the teachings in the prior art of the advantages of fusion proteins comprising a CBD binding domain and proteolytic cleavage site for ease of isolation of the heterologous protein and that plants represent an alternative expression system for the mass production of proteins.

Applicants traverse this rejection and withdrawal thereof is respectfully requested. As noted by the Examiner, Haynes et al. fails to disclose any fusion proteins expressed in transgenic plants or obtained from transgenic plants. However, more importantly, Haynes et al. does, in fact, not disclose or suggest the binding of a cellulose binding module (referred to as a polysaccharide binding peptide, or PBP) to a polysaccharide matrix, such that it can be readily released with mild, non-denaturing conditions. Haynes et al. discloses that "[t]he composition [containing the PBP ligand] may be removed from the oligosaccharide polymer with a removal

solution having low ionic strength, high pH or containing a chaotrophic agent." (Column 3, line 41-44) The only examples of such removal solutions disclosed in Hayes et al. are acid, base, urea, ethanol, DMSO, and the like (Column 26, line 16-17), and ethylene glycol (Column 31, line 65). However, these are all well-known denaturing agents. The present invention on the other hand, specifically requires the use of non-denaturing elution conditions.

In Shani et al., a plant is homogenized to bring the fusion protein into contact with the cellulosic matter to form a fusion protein-cellulosic matter complex (see Shani et al., col. 8, lines 49-56). As such, the fusion protein of Shani et al., binds to and becomes a part of the insoluble cellulosic matter complex. The process in Shani et al., thus seeks to employ precisely such CBM-s that would *not* be suitable in the method of the present invention. With the CBM containing fusion protein of the present invention, the CBM does not bind to insoluble cell-wall plant material. Thus, the instant invention employs an approach that is directly opposite to that of Shani et al., and the use of a CBM that does not bind to insoluble cell-wall plant material, and the advantage associated with this, is not suggested or taught by Shani et al.

As such, the instant invention cannot be achieved by the combined teachings of Haynes et al. and Shani et al. and there is further no suggestion or motivation to modify the reference teachings to achieve the instant invention. Withdrawal of the rejection is, therefore, respectfully requested.

In view of the above amendments and Remarks, Applicant believes the pending application is in condition for allowance.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact MaryAnne Armstrong, PhD, Reg. No. 40,069 at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §§1.16 or 1.17; particularly, extension of time fees.

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Respectfully submitted,

By _____

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ABSTRACT OF THE DISCLOSURE

The present invention relates to methods for protein purification of high-value heterologous proteins by providing fusion proteins suitable for affinity purification and improved and economical methods of proteolytic cleavage of fusion proteins. The methods of the invention are useful for large-scale production of purified recombinant proteins from plants, plant-derived tissue or plant cells. The invention aims to reduce the cost and improve the quality of downstream processing of heterologous proteins produced in plants and other biological production systems.